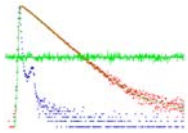


Förster resonance energy transfer (FRET)

The use of FRET has increased dramatically, especially in biological applications involving fluorescence microscopy. The main interest is the fact that this technique enables molecular interactions and conformational changes to be elucidated. Fluorescence energy transfer via the Förster mechanism occurs by a radiationless, coulombic, dipole-dipole interaction and is active over a range from 10 to 100Å. This makes it a well suited technique to probe nanoscale processes.

Resonance energy transfer

This coulombic, dipole-dipole interaction requires spectral overlap between the donor emission and an acceptor's absorption, along with the suitable orientation of their transition dipoles. It is manifest over the 10 to 100 Å range, which makes it well suited to follow molecular interactions and determine distances on the nanometre scale. This fact is made use of in microscopy to study interactions beyond the optical diffraction limit. Fig. 1 illustrates the possibility of non radiative resonance coupling (broken line transitions) from donor (D) to acceptor (A) as an alternative means for the donor to return to the ground state.

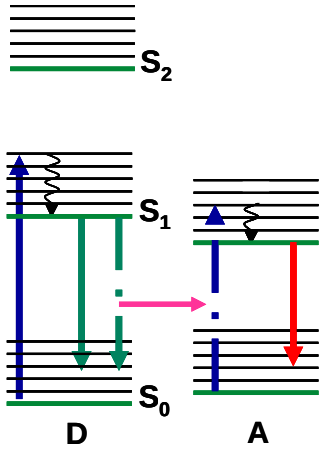


Fig. 1. Scheme for resonance energy transfer, shown using a Jablonski diagram. Apart from de-exciting via fluorescence, the donor has the possibility to transfer energy to the acceptor causing it to become excited.

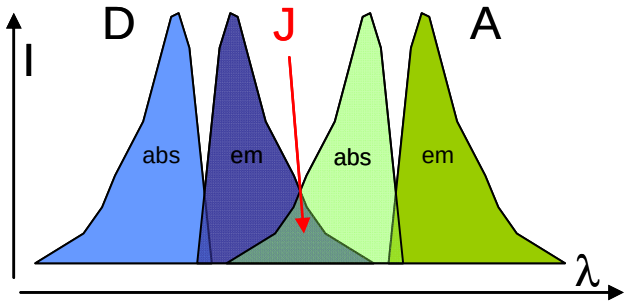


Fig. 2. Representation of the spectral overlap (J) of a donor (D) emission and acceptor (A) absorption.

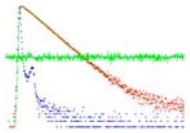
The strength of the spectral overlap (see Fig. 2.) is characterised by the overlap integral (J), which can be expressed as;

Overlap integral $$J = \int_0^\infty I(\lambda)\varepsilon(\lambda)\lambda^{-4}d\lambda$$

This contributes to the critical transfer distance, also known as the Förster distance (R_0), which is the distance at which energy transfer is 50% efficient and the formula is given below.

Förster distance $$R_0 = 0.2108\left[n^{-4}\phi_d\kappa^2 J\right]^{1/6}$$

Where; n is the refractive index, ϕ_d is the donor fluorescence quantum yield and κ is the orientation factor for the transition dipoles. For a random orientation κ^2 is $^2/_3$. This is the distance at which the probability of the excited state donor either transferring energy or emitting fluorescence (see Fig. 1.) is 50%.



The rate of energy transfer (k_{et}) can be expressed as,

Rate of energy transfer $$k_{et} = \frac{1}{\tau_d}\left[\frac{R_0}{R}\right]^6$$

τ_d is the fluorescence lifetime of the donor, without acceptor present. The above equation shows that there is a R^6 distance dependence on the rate of energy transfer, which effects the donor lifetime in the presence of the acceptor (τ_{da}). It is this R^6 dependency which gives the use of FRET its sensitivity. The measurement of the donor lifetime (in the presence of an acceptor) enables both the distance (R) and efficiency (E) of energy transfer to be determined; either relatively, if only the lifetime of the unquenched donor (τ_d) is known, or absolute, if the Förster distance (R_0) is also known for the particular system under study.

Efficiency $$E = 1 - \frac{\tau_{da}}{\tau_d}$$

Distance $$R = R_0\left[\frac{1}{E-1}\right]^6$$

The influence of the donor-acceptor distance on the donor lifetime and the efficiency of energy transfer is shown pictorially in Fig. 3.

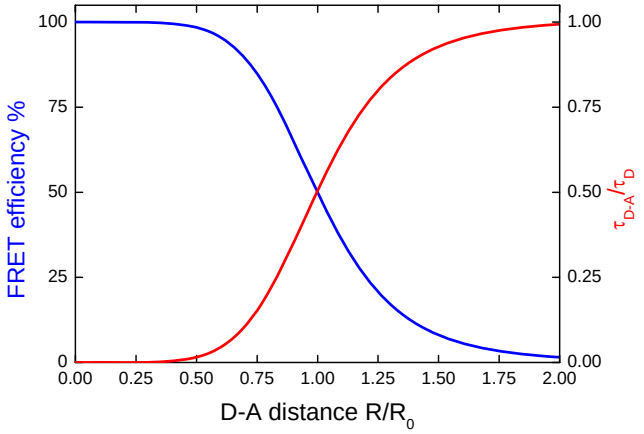


Fig. 3. Influence of donor-acceptor distance on the donor lifetime and FRET efficiency

This shows that the most sensitive distance region is 0.5 to 1.5 times the R_0 value.

Applications

Quite often there are recommended FRET pairs for specific applications. They may be tethered or free in solution. In the latter case it should be kept in mind that the solvent should be of sufficiently high viscosity to make the molecular diffusion length short in relation to R_0. This approach requires an excess of acceptors and the data can then be fitted to a form of stretched exponential. Adjustment can be made to include diffusion via Yokota-Tanimoto fitting. The optimum R_0 for a FRET pair is between 40 to 60Å to take advantage of its ability to sense nanoscale distances. However, it should also be kept in mind that not only distance information may be uncovered, since the orientation of the transition dipoles needs to be accounted for. Thus, interpretation of the results of a study should also consider if the relative orientation of the donor and acceptor changes as well as their distance.

Examples of some applications where FRET has found usage include; Polymers, model photosynthetic systems, characterisation of microheterogenous media, protein conformation, protein interactions and biosensors. In summary the main usage is where there is a requirement to measure changes in distance on the nanoscale, either within a macromolecule or by two molecules approaching and interacting.